FINAL PROGRESS REPORT

Simulated Space Radiation Studies for The Assessment Of Chromosomal Damage: An Integrated Experimental and Theoretical Approach

P.I. Aloke Chatterjee, Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, California

Co-P.I. Joel Bedford, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado

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Introduction

The research involves measurement of chromosome aberrations including lethal,non-lethal, potentially carcinogenic, and a class known as complex aberrations following irradiation of human cells with protons and HZE particles under different shielding conditions. Some fraction of the incident beam of high-Z particles undergoes nuclear fragmentation as they penetrate a thick shielding material. This creates components of the mixed beam of different radiation qualities. It is important to identify each of the components of the mixed beam for a better understanding of the overall chromosomal aberration yields. The experimental component of the research is complemented by research in developing a suitable theoretical model for interphase chromosomes in human nucleus to predict the radiation-induced aberration yields. These breaks are determined on the basis of biophysical and biochemical mechanisms of damage to DNA sites. In order to evaluate the chromosome aberrations an algorithm is being used which is based on a hypothesis that the probability of joining between any end of a double strand break with another is dependent upon the distance between them.

There are two main objectives of this research and they are: (1) experimental measurements of induced chromosomal aberrations by the mixed radiation field as a result of penetration of HZE particles through equivalent shielding materials as encountered in space travel and (2) development of a reliable theoretical model which can determine how cell killing and chromosomal aberrations vary as a function of the thickness and composition of radiation shielding. In the process of designing spacecraft for long duration space flights, NASA may have to consider many combinations of thickness and composition of the shielding materials and it may be impractical to make measurements for every such combination, hence the existence of a reliable theoretical model will be of great advantage in efficient spacecraft design.

In accordance with our objectives we have proposed the following specific aims that represent an integrated and interactive approach involving experimental and theoretical components of the project:

(1) Develop a suitable interphase chromosome model and calculate the radiation induced aberration frequencies.

(2) Measure the nuclear fragmentation spectra for incident HZE particles for different shielding materials and thicknesses and compare with existing semiemperical model on beam transport and make improvements in the model if needed.

(3) Integrate the interphase chromosome model and the nuclear fragmentation spectra to estimate and predict the aberration frequencies as a function of incident ion and shielding material and thickness for mixed radiation fields.

(4) Measure chromosomal aberrations, including (a) lethal, (b) non-lethal and potentially carcinogenic, and (c) complex aberrations following irradiation of G_0 human normal fibroblasts(or in some cases epithelial cells) over a range of doses from helium ions and HZE particles under shielding conditions as described in aim 3. Measured values would be compared with predictions of the model. Starting with part (a) dicentrics, centric rings, interstitial and terminal deletions would be measured by classical scoring criteria. Independent measurement of the cell survival colony formation would be made. From the same irradiated samples part (b) would proceed to measure stable symmetrical translocations by 1 or 2-

color FISH whole chromosome painting. Also a fraction of complex aberrations would be recorded. For part (c) a select few especially interesting samples would be chosen to measure complex aberrations by mFISH and estimate the fraction of aberrations that are complex for the different beam qualities.

Chromosome aberrations are considered as surrogate end points for carcinogenesis. Such measurements in the blood lymphocytes of eight crew members were reported in the literature.This research directly contributes to at least two questions in the Critical Path Roadmap. One question relates to 10.08 under "Carcinogenesis caused by radiation(38)" which states "how do the thickness,design,and material composition of space vehicles affect the internal radiation environment and biological assessment"?. The second question relates to 10.09 which states that " What are the cancer risks in humans for spaceflight"?

Chromosomal aberrations figure prominently in the pathways of damage development leading to most of the important biological effects of ionizing radiation exposure, including cell killing,intragenic mutations, and cancer. Aberrations involved in cancer may be produced directly in the first cell cycle after exposure or can arise in later generations asa consequence of induced genomic instability.

Most chromosomal aberrations are "exchange types". These are much more frequent than terminal deletions that result from a break in chromatin of an interphase cell that simply does not rejoin. Two or more breaks must occur within some proximity in order to mis-rejoin to form the aberration, and the density of ionizations along the tracks of the charged particle delivering the dose greatly influences the frequency per unit dose of such "clustered" damage events, and thus, the RBE for different qualities of radiation. At one extreme are energetic electrons that deposit the dose from high energy X- or gamma radiation. Here,perhaps only a small part of the biological effect might result from single electron tracks. At other extreme are slow heavy charged particles such as low-energy alpha particles with very short secondary electron delta rays associated with the track. Biological effects of HZE particles do not show the relatively simple picture illustrated by the extremes, and LET vs. RBE relationships are not necessarily predictable by simple interpolations between these extremes.

Although high-energy heavy ions comprise only a small fraction of the charged particle flux in the GCR, their large energy deposition makes them an important contributing factor to the health risk for crew -members on long duration space missions. Because the radiation dose per particle is approximately proportional to the square of the particle charge, heavy ions contribute significantly to the radiation dose received by humans in space. Crews on lunar and mars exploration missions will be exposed to the full GCR flux, modulated by the solar wind and also modified by spacecraft shielding. In order for NASA to have accurate knowledge of crew exposures, the radiation fields inside spacecraft must be evaluated through a combination of measurements and theoretical modeling. Theoretical models of biological responses may allow to accurately assess the health risks associated with these exposures.

A breakdown of a heavy nucleus occurs in nucleus (projectile) – nucleus (target) collisions. These can be grouped into two broad and not entirely distinct categories: (1) peripheral interactions and (2) central collisions. In peripheral interactions, a small number of nucleons (such as protons and neutrons) is removed from the projectile ion. The remnant of the projectile continues forward, in nearly the same direction as the

incident ion, with approximately the same velocity. Lighter fragments-mostly protons and helium are also peaked in the forward direction but they have a wider angular distribution than the projectile fragments. In central collisions, which are less common than the peripheral collisions, the projectile ion is entirely broken up into a spray of light particles and some may emerge at considerable angles with respect to the trajectory of the incident ion. Regardless of the type of collisions, all emerging fragments are lower in charge and mass than the incident particle, and because the fragments approximately maintain the velocity of the incident ion, they will penetrate to depths that exceed the range of the incident ion.

We measured dose-responses for chromosome aberration induction in low passage normal human fibroblasts in the first mitosis following irradiation of non-cycling G0 cells with HZE iron ion beams of various energies and after filtration with various thicknesses of aluminum or polyethylene. Aberration yields over a range of doses in several independent experiments at the NSRL facility at Brookhaven National Laboratory for a given set of conditions were not significantly different and the pooled data for the different energies and different thicknesses of shielding indicated no significant effects of shielding on the relative effectiveness for aberration induction. By comparison with measurements carried out in parallel using gamma rays, an RBE of about 4.1 was estimated at the level of effect of 1 aberration per cell for all the Fe ion initial energies (0.3, 0.6, and 1.0 GeV/n) and for all the shielding thicknesses tested for the 1.0 GeV/n and 0.6 GeV/n iron ion beams.

Materials & Methods

Experimental Description:

We irradiated cells at Brookhaven National Laboratory to make a preliminary assessment of the effectiveness of 1Gev/n iron ions in producing chromosomal aberrations over a range of doses relative to the corresponding doses of gamma-rays required to produce the same levels of effect. An additional aim was to compare the dose response for aberration induction with the cultured normal human fibroblasts we have chosen for this study with results we have obtained in previous experiments and conditions using other strains of human fibroblasts.

The experimental plan and set-up was as follows:

1) Approximately 8 days before transporting cultures to Brookhaven, we inoculated T25 polystyrene flasks with 1.5x10exp5 GM2149 low passage normal human fibroblasts. After 2 days the medium was changed, the flasks were completely filled with medium, and the cells were allowed to continue growth to form a confluent monolayer of cells.

2) Before sending the cultures to Brookhaven, BUdR was added to the flasks to a final concentration of 10 mMolar and the flasks were sealed with paraffin wax. The purpose of this addition was to enable the detection of any cells that may have been have been cycling at the time of (or after) the radiation exposure. The presence of cycling cells would hopelessly complicate the interpretation of results, since we would

then not know the cell cycle stage at the time of irradiation and would not know whether the cells were in the first, second, or even third post-irradiation cell division at the time mitotic cells were prepared for analysis.

3) Cells were shipped to Brookhaven and kept unopened in a 37 C. incubator until they were irradiated with 1Gev/n iron ions in the flasks. Doses of 0, 0.3, 0.6, 0.9, 1.2, and 1.5 Gy were administered. With the geometrical orientation of the cell nuclei in the plane of the flask surface, and the beam at right angles to this surface, the 1.2 Gy dose would result in an average of approximately 1 iron ion traversal per cell nucleus. After irradiation, the flasks were returned to the incubator to allow full processing of radiation damage, i.e., they were not sub cultured immediately, but were then shipped back to Colorado State University. This treatment does not decrease the cell survival or increase the aberration frequency relative to cultures that were set up at the same time but were kept at CSU.

4) A parallel set of cultures was set up for gamma ray exposure, but upon arrival at Brookhaven the gamma source was out of service, so we had to rely on utilization of an x-ray machine and dosimetry that turned out to be very much inaccurate for the exposure conditions that were used. This was apparent in the chromosome aberration results. This "biological dosimetry" indicated that actual x-ray doses delivered were only 40% of the intended doses. This estimate was made with the following assumptions. Since the aberration yields for 1Gev/n iron ions agreed very closely with previous runs it seemed fair to assume that the different cell strains used and cell irradiation and handling conditions were not a variable, so comparing the dose response for x-rays using the data from previous runs with that from the current run required a dose modifying factor of 2.5 (current run nominal doses multiplied by 2.5) to bring the current aberration yields into agreement with those obtained previously at Brookhaven. These also agree with several other measurements of aberration yields for x- or gamma irradiations of these cells with allowance for an RBE of about 0.9 for gamma rays relative to x-rays of the energy used here.

4) After the cultures were returned to CSU, samples were sub-cultured to lower cell densities to set up several cultures to be sampled at various times when the wave of first division mitotic cells were expected. This differed somewhat depending on the dose. For the collections, Colcemid was added to the first samples at 24 hours after sub-culture and the harvest was at 30 hours. For the second collection the sampling was from 30 to 36 hours; for the third, from 36 to 42 hours, and for the fourth, from 42 to 48 hours.

5) After collection and fixation, cell suspensions were kept in fixative in the freezer and slides were prepared from these suspensions as needed. Cells can be kept for long periods of storage this way, and we still have cells that can be used for FISH chromosome painting of particularly interesting samples.

6) One series of slides was prepared and stained using anti-BUdR antibody to determine whether a significant proportion of cells had "cycled" from the time the cultures left CSU until they arrived back at CSU. There were, in fact a significant proportion that cycled, but these were eliminated from the scoring of mitotic cells, i.e., only cells that had not cycled (had not incorporated BUdR) were included in the scoring. We are fairly sure the cycling occurred between the time they left CSU and before they were irradiated, since it appeared from examination of the flasks before they left that they were not "maximally" confluent. While this did not affect the outcome since we were

able to confine the assay to the desired cells, in the next run we plan to allow at least 2 more days in the "confluent" state before sending cultures to Brookhaven. Further, we will probably want to add the BUdR to the cultures at Brookhaven shortly before irradiation. The reason for this is that when a significant proportion of cells have cycled it complicates the scoring because each and every cell must be identified for the absence of BUdR before scoring it for aberrations. If frequency of cells without BUdR was 3% for example, inclusion or exclusion from the scoring would not appreciably affect the result, but if the frequency was 30% it would be particularly important to exclude these cells. This was the case for some of the samples in the present run.

Chromosome spreads were prepared and after aberrations were scored the identity of the samples is decoded and the results analyzed. Acentric fragment producing aberrations include dicentrics with an accompanying acentric fragment (=1 aberration), interstitial intra-arm deletions (acentric rings), centric rings, and terminal deletions, which by convention we consider to be acentric fragments that are not rings but are longer than the width of a chromatid. We did not verify the presence or absence of telomeres on acentric fragments but the scoring criteria were uniformly applied on all samples, and these samples were coded as mentioned above.

The thicknesses and composition of the beam shielding (filters) are summarized in table 1.

Table 1

^{56}Fe ion Energies Gev/amu	**Filtration Materials and Thicknesses (cm)**	
	Aluminum	**Polyethylene**
1.0	**0**	**0**
	1.8	**0**
	5.4	**0**
	0	**5.5**
	0	**11.0**
0.6	**0**	**0**
	1.8	**0**
	5.4	**0**
	0	**5.5**
	0	**11.0**
0.3	**0**	**0**

Theoretical Description:

Simultaneous to experimental efforts we have started the theoretical model development aspect of the project during the last fiscal year. There are four essential components of our model which are: (i) track structure of HZE particles which include the high energy delta ray tracks, (ii) modeling of interphase chromosomes,(iii) packing of the 46 human chromosomes within the nucleus, (iii) induction of damage to various DNA sites by biophysical and biochemical mechanism and (iv) an algorithm which uses a proximity function to estimate the “faithful” and “unfaithful” rejoining of double strand breaks. The

proximity function algorithm has been used in the past by us for low-LET radiation exposures with good results but has never been tested for HZE particles. During the last year we have extensively tested the algorithm for such radiation qualities and there are good indications that this approach will be valid also in this case. The overall algorithm, which is still under further development, essentially identifies the double strand breaks and their individual locations within the genome and then by using the proximity function algorithm calculate the various types of chromosome aberrations. In our model the number of dicentrics produced equal the number of reciprocal translocations. Such results have been validated for low-LET radiation and we are in the process of comparing with our initial experimental data that has become available from measurements at Brookhaven AGS. Experimental data on chromosomal aberrations have been obtained between 0 and 1.5 Gray and within this range our theoretical calculations agree reasonably well with the data except at 0.9 Gray. We are examining the reason for this difference and our initial finding indicates that the experiment at this particular dose has to be repeated. Experiments are also needed at higher dose as well in order to compare with the current predictions of the theoretical model.

Results

The chromosomal aberration frequencies in low passage normal human GM2149 mitotic cells in their first post-irradiation mitosis, after irradiation with 1Gev/n iron are summarized in table II.

TABLE 1I

Radiation Type	Dose (Gy)	#Cells Scored	Cells without Aberrations (%)	Chromosome-type Aberrations per cell				
				Deletions*	Dicentrics	Centric Rings	Total	Net
1Gev/n Iron	0	158	99.4	0	0.006	0	0.006	0
	0.3	98	76.5	0.143	0.122	0	0.265	0.259
	0.6	67	71.6	0.104	0.209	0	0.313	0.307
	0.9	32	62.5	0.438	0.125	0	0.563	0.557
	1.2	57	36.8	0.404	0.544	0.018	0.966	0.960
		20	26.9	0.650	0.700	0.050	1.450	1.440

* *Deletions include interstitial and terminal deletions or acentric fragments not associated with dicentrics or centric rings.*

The corresponding results for x-ray exposures, with biological dosimetry as explained above are sown in table III

TABLE III

Radiation Type	Dose (Gy)	#Cells Scored	Cells without Aberrations (%)	Chromosome-type Aberrations per cell				
				Deletions*	Dicentrics	Centric Rings	Total	Net
X-rays	0	160	97.5	0.013	0.013	0	0.026	0
	0.48	218	95.9	0.018	0.023	0	0.041	0.015
	0.96	193	91.2	0.067	0.031	0.01	0.108	0.082

	1.44	97	78.4	0.155	0.113	0	0.278	0.252
	1.92	100	73.0	0.200	0.130	0	0.330	0.304
	2.40	114	67.5	0.237	0.149	0	0.395	0.369

[*] *Deletions include interstitial and terminal deletions or acentric fragments not associated with dicentrics or centric rings.*

For purposes of comparison with a previous "Brookhaven run" but with AG1521 low passage human fibroblasts irradiated to a dose of 1.25 Gy with 1Gev/n iron ions the frequency of dicentrics in the first post-irradiation mitosis was 91 dicentrics in 171 cells scored or 0.53/cell. one dicentric was observed in 342 control cells in this experiment. For 5 Gy of gamma rays in 100 cells scored, there were 57 dicentrics or 0.57/cell.
In yet another previous experiment with gamma rays and using AG1521 cells, with 100 cells scored per sample, doses of 2 Gy and 6 Gy, yielded dicentric frequencies of 0.17/cell, and 0.93/cell, respectively.
A comparison of the theoretical results with the available experimental data (figure 1) clearly demonstrate that the proximity function algorithm will be appropriate for rejoining of double strand breaks produced by HZE particles which may have very important implications regarding the complexities of damages produced by these types of radiation qualities. We are investigating the implications. Our current theoretical results depend upon two important parameters and they are: (i) an overlap parameter which considers an average overlap between chromosomes in the nuclear organization and (ii) a range parameter which is associated with the critical choice of a distance between the double strand breaks. As far as the overlap parameter is concerned we think that the chromosomes are more or less occupy discrete locations in the nucleus with very little(7%) overlap between them. For the range parameter we find that most of the experimental data are in good agreement when we take this as 500 micro-meter for the human GM2149 fibroblasts. Interestingly enough this value was the same for the human lymphocytes.

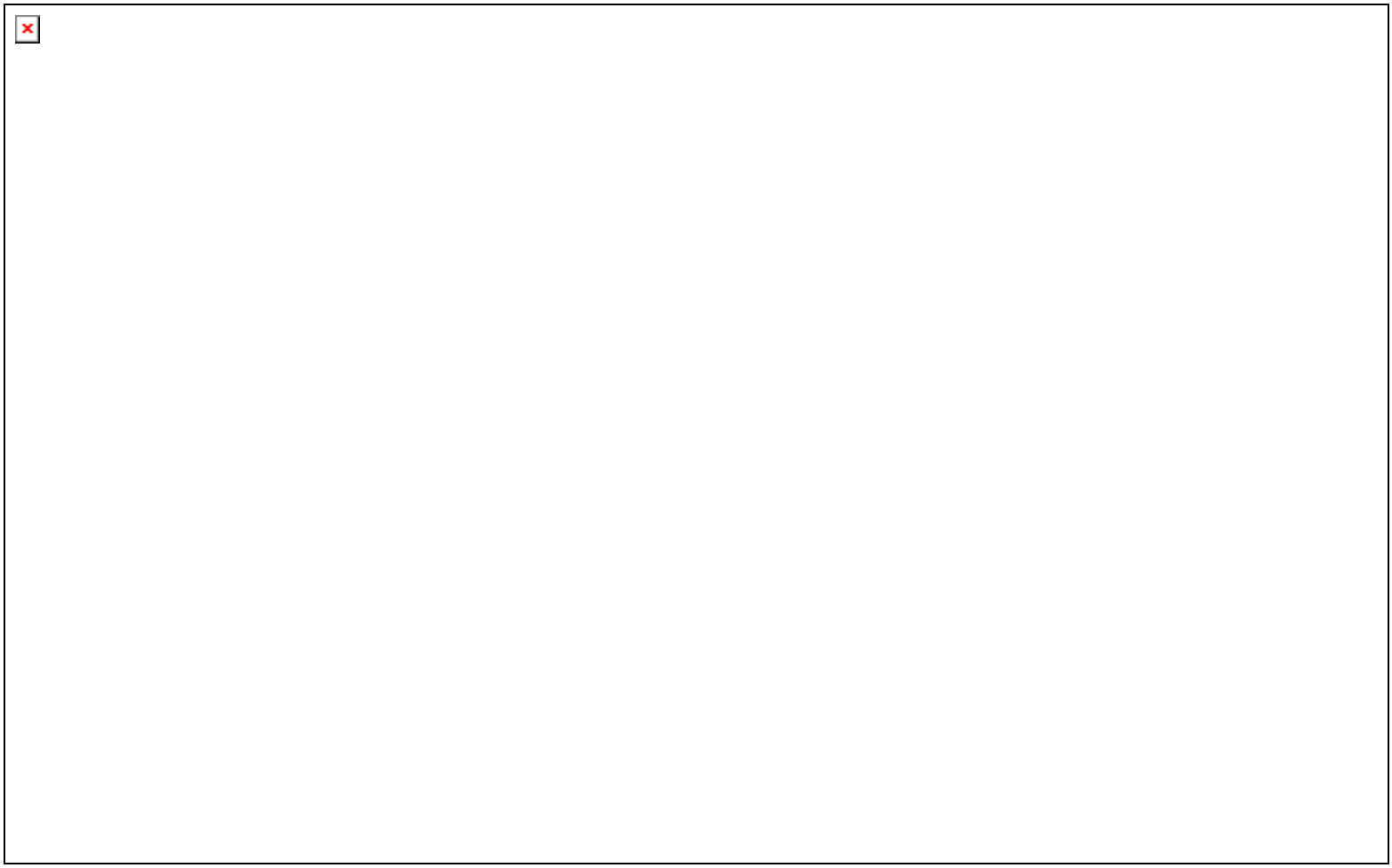

Figure 1

Shielding Material : Experimental Results and Comparison With Theory

In these measurements we had introduced shielding materials and wanted to evaluate the effects of nuclear fragmentation. Using 1 GeV/n Iron particles, we have irradiated GM2149 normal human fibroblasts in G0 and then fixed them when cells reached their first post irradiation mitosis. Prior to fixing, the cells were allowed to undergo full damage processing. 1.8cm of Aluminum or 5.5 cm of polyethylene were used as shielding materials to create mixed radiation fields. Based on fragmentation measurements using a stack of silicon detectors, about 20% of the primary ions seemed to be involved in the charge changing nuclear reactions in the Aluminum target and about 45% were involved in the polyethylene target. The particle distribution measured behind the targets have been used as input spectra in the theoretical calculations. Experimental measurements of chromosomal damage included deletions, dicentrics, centric rings and total aberrations. In the case of Aluminum, nuclear fragmentation does not seem to influence the aberration data. And in the case of polyethylene, there seem to be some effect on the aberration data due to nuclear fragmentation. Theoretical model

calculations were executed using the same model for chromosome aberrations as used with no shielding situation. A comparison of the theoretical results with the experimental data with or without shielding demonstrated excellent agreement(figure 2). Furthermore the two parameters that were determined from the fall 2002 experiments remained the same which was encouraging and provided a better confidence in the theoretical modeling. These results were presented in the NASA workshop meeting in April 2004.

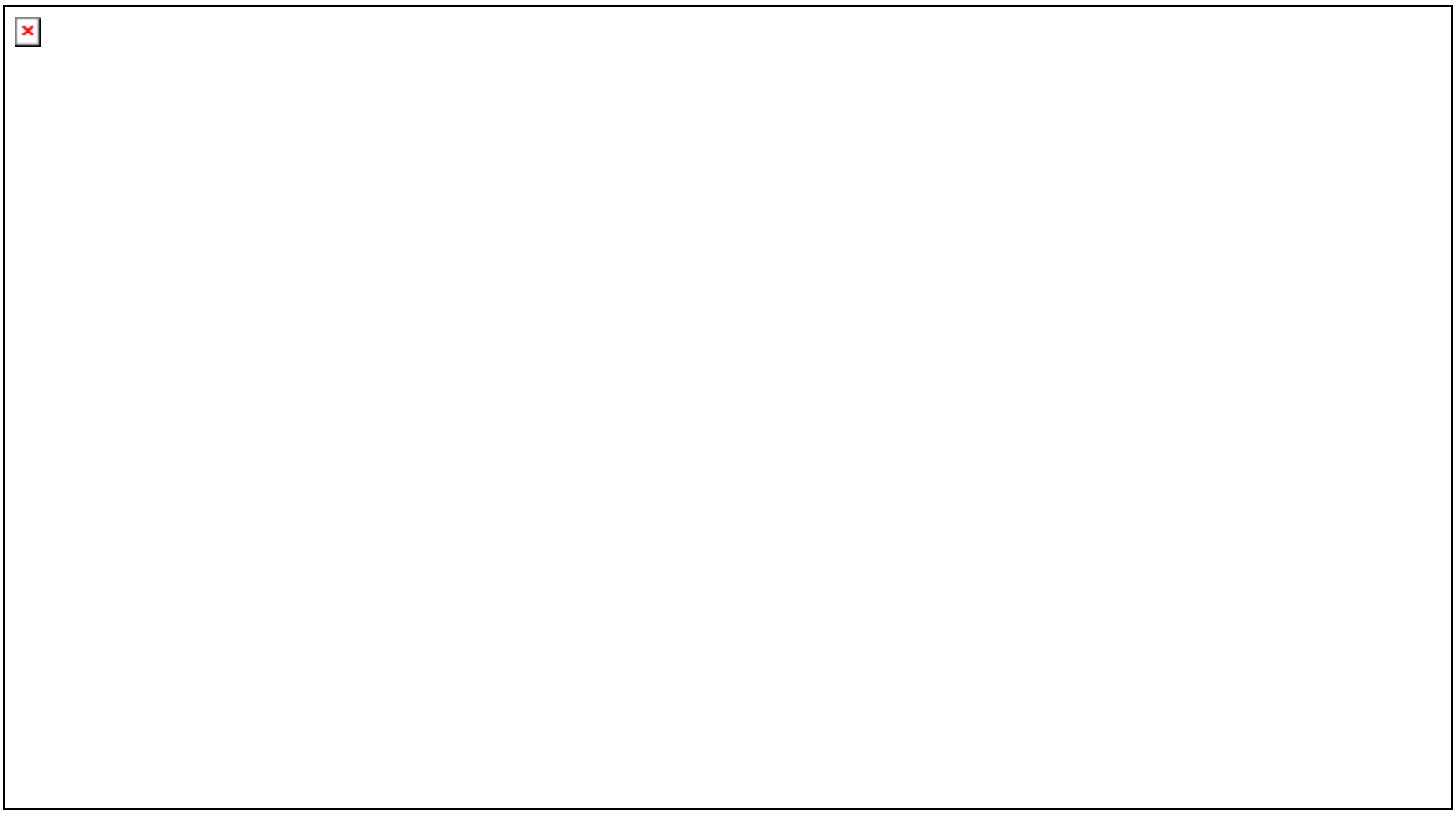

Figure 2

The theoretical curves have been calculated using the chromosome model for pure beams. The upper curve in red is for 100% iron particle as if the primary ion did not fragment(hypothetical) at all even though the beam has penetrated through 5.5 cm of polyethylene. The lower curve in blue is for 55% of iron flux and does not contain any contribution from the nuclear fragments. The experimental data agree very well with the blue curve even though the fragments have not been included. This implies that the contributions from the fragments are not appreciable to make any difference. This was somewhat of a surprising result. We are in the process of making new measurements by increasing the shielding thicknesses and repeat the model calculations and determine to what extent the fragments influence the overall data.

Approximately 7700 mitotic cells were scored for the pooled results shown in the figure 3. Results were obtained in 5 separate NSRL runs at BNL; NSRL 1,2,4,5, and 6, and for each run, repeats of at least some runs under the same conditions were carried out as an indication of the consistency of results from one run to the next. In fact, the results from one run to the next for a given set of conditions did not differ significantly, so the data for each condition were pooled and are summarized in the figure3. Total aberration frequencies observed are plotted, but the general conclusions for different types of aberration was similar. The breakdown of aberration types are also available The error bars shown (when larger than the plotted points) are standard deviations calculated from mean values assuming a Poisson distribution of aberrations among cells. The observed proportions of cells without visible aberrations were in good agreement with the calculated Poisson expectations based on the observed mean values. Curves were fitted by a least-squares method. A linear quadratic function provided a significantly better fit than a simple linear function of dose for the gamma-ray data, but not significantly better for the HZE Fe ion data. The RBE from these fits at an effect level of 1 aberration per cell was 4.1

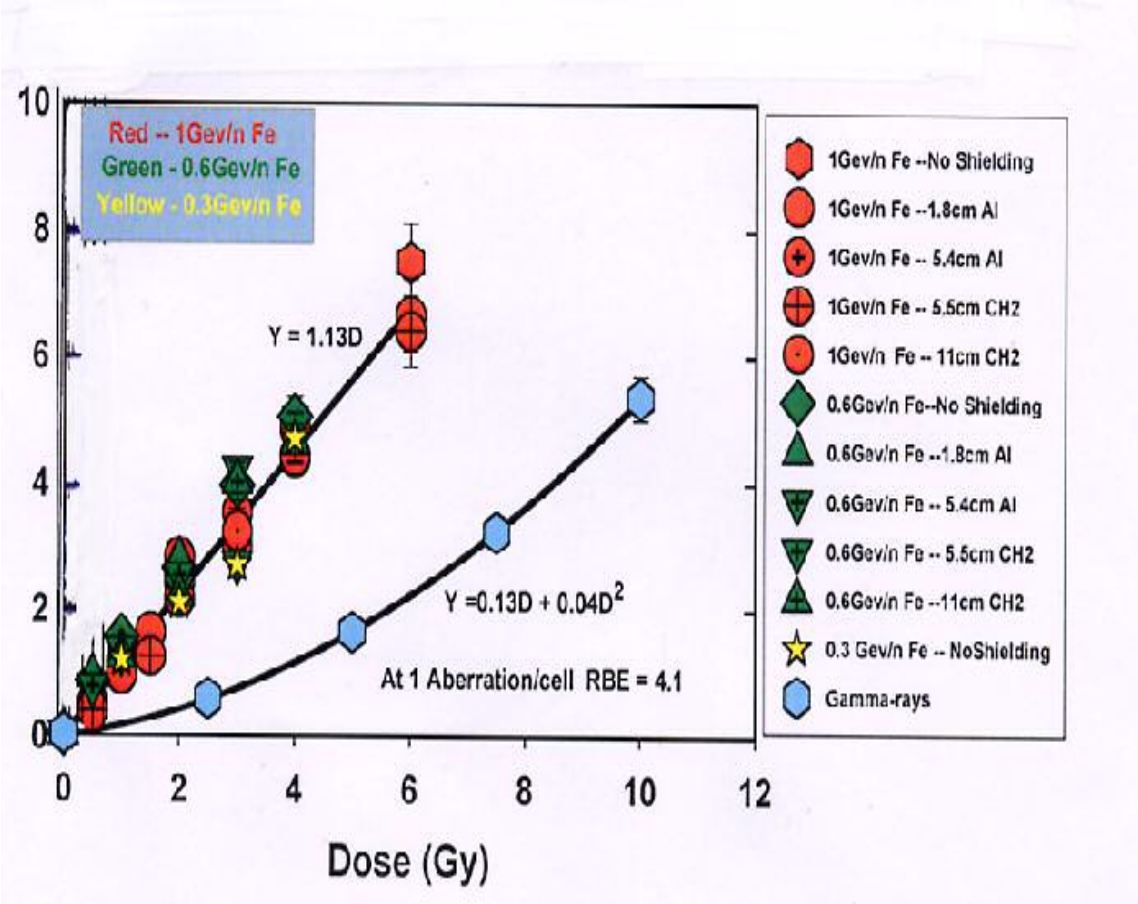

Figure 3

Discussion:

It is clear from the Critical Path Roadmap that in order to understand risks associated with HZE induced carcinogenesis we must understand the radiation environment in which the astronauts will be operating inside the spacecraft. This radiation environment is dependent upon: (i) all the energetic components in the galactic cosmic rays and (ii) all the lower atomic number nuclear fragments that are generated as a result of nuclear disintegration as a single component of GCR penetrates through the shielding materials. The overall situation due to both of these sources of radiation is to present a very complex radiation environment. In order to estimate the carcinogenic potential of these radiation environments a systematic approach is required. This proposal relates to the effects of the fragments produced by the shielding. Once we understand the biological effects of the mixed beams acting simultaneously on cellular targets and consequent cellular and tissue responses, the approach could easily be extended to the situation where the incident radiation prior to the interaction with the shielding materials is a mixture of various radiation qualities as to be encountered in deep space. The theoretical model developed in this project can be used to predict biological effects such as chromosome aberrations for incident mixed beam combined with mixed beam due to nuclear disintegration caused by shielding materials. The effectiveness of the HZE iron ions for induction of acentric fragment producing chromosome-type aberrations at the first mitosis after irradiation of human GM2149 cells in G0 was not measurably different for incident Fe ion energies of 0.3, 0.6, or 1GeV/n. Neither did the filtration of 1 GeV/n or 0.6 GeV/n Fe ions with 1.8cm or 5.4 cm of aluminum or 5.5cm or 11.0cm of polyethylene measurably alter the effectiveness of the emerging beam. One of the most important conclusions derived from the successful completion of this project is that based on our current estimate of thicknesses of shielding materials, the biological effects of a mixed beam due to fragmentation processes will not be much different than the un-fragmented and pure beam.

List of Presentations

1. High-LET Radiation Hazard Associated With Deep Space Travel—Invited lecture in the Annual Radiation Research Society Meeting in the Year 2004.
2. Chromosome Aberrations: Experimental Measurements and Theoretical Model—Presented at the NASA's annual meeting in the year 2004.
3. Collaborative Research With Williams R.Holley in Developing Theoretical Models in Radiation Biology- Invited Lecture in the 14^{th} International Symposium on Microdosimetry in the Year 2005.
4. The RBE of HZE Fe Ions and Effects of Aluminum and Polyethylene Shielding: Chromosomal Aberration Induction in Normal Human Cells in Culture- Presented at the International Space Radiation Meeting in Moscow in the Year 2006.